PRODUCT INFORMATION & MANUAL

Aspartate Aminotransferase Activity Assay Kit (Fluorometric)

NBP3-24471

For research use only.
Not for diagnostic or therapeutic procedures.

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Aspartate Aminotransferase Activity Assay Kit (Fluorometric)

Catalog No: NBP3-24471

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Fluorimetric Microplate Reader

Sensitivity: 0.03 U/L

Detection range: 0.03-1.5 U/L

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 7.3

Average recovery rate (%): 100

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure aspartate aminotransferase (AST/GOT) activity in cell, animal tissue, serum (plasma) and other liquid samples.

▲Detection principle

Aspartate aminotransferase (AST/GOT) is an important indicator of liver inflammation. When cells are damaged, the permeability of cell membrane increases, resulting in the release of AST in the cytoplasm into the blood and the increase of serum AST activity. AST can catalyze the substrate to produce pyruvate. Pyruvate is oxidized to produce hydrogen peroxide, which causes the fluorescent probe to produce fluoresce. The activity of AST can be calculated by measuring the increase of fluorescence value at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Substrate	1.5 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Aqueous Alkali	0.6 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 5	100 mmol/L Pyruvate Standard	1 mL × 1 vial	-20℃ , 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Micropipette, Vortex mixer, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/587 nm).

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

Prevent the formulation of bubbles when the supernatant is transferred into the microplate.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 3 working solution:

   Dissolve a vial of reagent 3 with 1.2 mL of reagent 1 fully and preserve it on ice for use. The prepared reagent 3 working solution can be stored at -20°C for 1 week.
3. Preparation of reaction working solution:

   Mix the reagent 1, reagent 2, reagent 3 working solution and reagent 4 at a ratio of 50: 25: 20: 5. Prepare the fresh solution before use and stored with shading light. The prepared solution should be used up within 1 h.
4. Preparation of 1 mmol/L pyruvic acid standard stock solution:

   Take 10 μL of reagent 5 to 990 μL of reagent 1 and mix fully. Prepare the fresh solution before use.
5. Preparation of 100 μmol/L pyruvic acid standard solution:

   Dilute 1 mmol/L pyruvic acid standard stock solution with reagent 1 at the ratio of 1: 9 and mix fully. Prepare the fresh solution before use.

▲ Sample preparation

1. Serum (plasma) and other liquid sample:

Detect the sample directly. If the sample contains a lot of lipids, chylous, etc., it can be centrifuged at 5000 g at 4°C for 5 minutes, take the clarified part and store it on ice for testing.

2. Animal tissue sample:

Accurately weigh the tissue sample, add 9 times the volume of reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add reagent 1 at a ratio of cell number (10^6): reagent 1 (mL) =1: 1. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.03-1.5 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat heart tissue homogenate	300-600
10% Rat brain tissue homogenate	300-500
10% Rat liver tissue homogenate	200-500
Human serum	5-15
10% Rat kidney tissue homogenate	200-500
Rat serum	10-20
10% Rat spleen tissue homogenate	50-100
293T cell(1×10^6)	10-20
10% Mouse lung tissue homogenate	100-200
293T cell supernatant	5-15

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 100 μmol/L pyruvic acid standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 80, 60, 50, 40, 30, 20, 10, 0 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	100 μmol/L pyruvic acid standard (μL)	Reagent 1 (μL)
A	0	0	200
B	10	20	180
C	20	40	160
D	30	60	140
E	40	80	120
F	50	100	100
G	60	120	80
H	80	160	40

2. The measurement of samples

(1) Standard well: Add 20 μL of standard with different concentrations into the corresponding well.

Sample well: Add 20 μL of sample into the corresponding well.

(2) Add 100 μL of reaction working solution to each well.

(3) Mix fully with microplate reader for 5 s and stand at room temperature for 3 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, recorded as F_1, and then react at room temperature for 60 min with shading light. The fluorescence intensity of each well was determined under the same wavelength, and recorded as F_2, then $\Delta F = F_2 - F_1$ (Note: There is no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of $F_{2(standard)}$).

▲Summary operation table

	Standard well	Sample well
Sample (μL)		20
Standard with different concentrations (μL)	20	
Reaction working solution (μL)	100	100
Mix fully and stand at room temperature for 3 min. Measure the fluorescence intensity of each well, recorded as F_1, and then react at room temperature for 60 min with shading light. The fluorescence intensity of each well, and recorded as F_2, then $\Delta F = F_2 - F_1$ (Note: There is no change in fluorescence value of standard well, plot the standard curve with the fluorescence value of $F_{2(standard)}$).		

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

1. For serum (plasma) sample:

Definition: The amount of enzyme in 1 L of serum or plasma that catalyze the production of 1 μmol pyruvic acid per minute at 25°C is defined as 1 unit.

$$\text{AST activity (U/L)} = (\Delta F - b) \div a \div T \times f$$

2. For tissue and cell samples:

Definition: The amount of enzyme in 1 g of sample protein that catalyze the production of 1 μmol pyruvic acid per minute at 25°C is defined as 1 unit.

$$\text{AST activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

Note:

y: $F_{Standard} - F_{Blank}$ (F_{Blank} is the fluorescence value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: The absolute fluorescence value of sample, $\Delta F = F_2 - F_1$.

T: The reaction time, 60 min.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in sample, gprot/L.

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Appendix I Data

▲Example analysis

For 10% rat spleen tissue, dilute for 80 times, take 20 μL for detection, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 96.281 x + 202.56, the average fluorescence value of the sample F_1 is 1163, the average fluorescence value of the sample F_2 is 9155, the concentration of protein in sample is 6.90 gprot/L, and the calculation result is:

AST activity (U/gprot) = (9155 - 1163 – 202.56) ÷ 96.281 ÷ 60 × 80 ÷ 6.90

= 15.63 U/gprot